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NOTE

Bdelloid Rotifers (Rotifera: Bdelloidea) Inhabiting Larval Black Flies (Diptera: Simuliidae) and Their Effect on Trichomycete (Zygomycota) Fungal Abundance

Black fly larvae (Diptera: Simuliidae) are restricted to lotic habitats where they anchor themselves with a silken pad spun onto solid substrates (e.g., rocks) and filter food from the water column (Adler and McCreadie 1997). Symbiotic relationships have evolved between black flies and other organisms, including, bacteria, fungi, nematodes, viruses, and protozoans (Crosskey 1990). During laboratory investigations of endosymbiotic trichomycete fungi of the genus *Smittium* (Zygomycota: Trichomycetes) and the larval black fly *Simulium vittatum* Zetterstedt cytospecies IS-7, we found bdelloid rotifers (Rotifera: Bdelloidea) in the simuliid larval midgut (Fig. 1). Here we report the first record of this association and provide evidence that the presence of rotifers in the larval midgut influences the ability of *Smittium* to establish in the hindgut.

Larvae of *Simulium vittatum* cytospecies IS-7 were reared from eggs obtained from a parasite-free colony housed at the University of Georgia (Athens, GA, U.S.A.). Approximately three weeks after submergence of eggs in 500 ml of aged tap water maintained at 22°C (Percival® incubator, Model: I-36 VL), 40 larvae each were placed in 1-L polypropylene containers with 500 ml of aged tap water and moved to another incubator. All containers were aerated with aquarium pumps, and larvae were fed daily on a fish food slurry (McCreadie and Colbo 1991).

Fungi were reared on plates of 1/10 Brain Heart Infusion agar (Difco® 235–500: 0037-17) at room temperature (22–25°C) with sterile water overlays added to induce trichospore production. Trichospores are single sporangia, each housing a single sporangiospore and are the asexual infective stage of trichomycete fungi (Lichtwardt 1986). Once the trichospore enters the black fly larval hindgut, the sporangiospore extrudes, attaches to the cuticle, and produces a new thallus. In our experiments, a dosage of 4,000 trichospores/ml of rearing water was used.

To determine fungal abundance in hosts, larvae 4 days after inoculation were dissected in a drop of distilled water and the mid- and hindguts removed. Under phase-contrast microscopy, the hindgut was viewed at 400× through a 10 mm × 10 mm ocular grid. The number of grid squares that contained one or more hyphae were counted; relative abundance was expressed as the percentage of grid squares containing hyphae. During routine dissections in three experiments, four different treatment containers, out of 36, had larvae with active bdelloid rotifers in their midguts. In experiments 1 and 2, one out of 12 containers in each experiment had larvae with rotifers; in experiment 3, two out of 12 containers housed infected larvae. A total of 186 larvae were examined from these containers and 37 (19.9%) contained rotifers; abundance of bdelloids ranged from 0 to 24 individuals per larval host. Rotifers used a telescoping-type locomotion and fed on green algae in the simulid midgut. Although the identity of the rotifers remains unknown, they possess characters consistent with the family Philodinidae (Wallace and Snell 2001). Whether the black fly larvae acquired bdelloids before or after trichospore inoculation, is unclear.

Bdelloid rotifers are free-living invertebrates that inhabit aquatic vegetation, sediment of lentic habitats, moist forest soils (Wallace and Snell 2001), and even the surface of freshwater insects and crustaceans

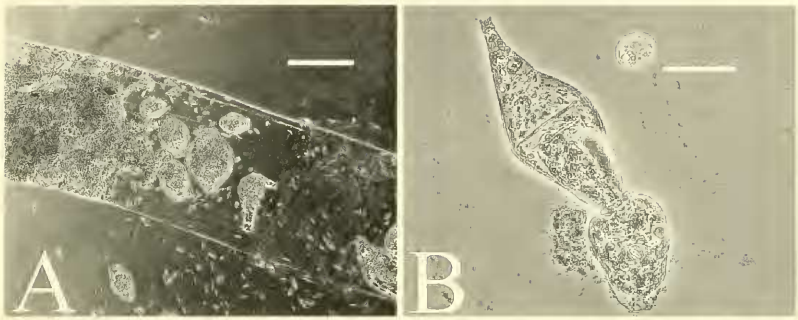

Fig. 1. A, Bdelloid rotifers inhabiting the peritrophic matrix of a larva of *Simulium vittatum* cytospecies IS-7. Bdelloids are located posterior to the food bolus on the left. Scale bar = 100 μm. B, A bdelloid rotifer with partial eversion of internal viscera. Scale bar = 25 μm.

(Pennak 1978). There are two dubious reports of bdelloid rotifers entozoic in larval *Culex* (Diptera: Culicidae) and *Chironomus* (Diptera: Chironomidae) (Marchoux 1898, Bartos 1951). Relationships between rotifers and trichomycete fungi are unknown, but some parasitic fungi, such as *Harposporium* (Deuteromycota: Moniliales), depend on rotifers for reproductive success (e.g., Baron 1980).

The source of the bdelloids in our material is unknown but might be related to the anhydrobiotic nature of these rotifers (Ricci 1987, 1998). The ability to survive dehydration (i.e., in a dessicated form) might indicate an airborne origin from within the laboratory or building. Stock cultures of larvae were always free of rotifers. Infections were not a result of epizoic rotifers since they were contained between food boluses in the black fly midgut in three different experiments conducted on different occasions. Thus, our reported occurrence of rotifers is not an isolated event. Bdelloid rotifers were noted in hindguts of several larvae, but none were alive. Also, dead bdelloid rotifers have been seen in hindguts of field collected *S. tuberosum* (Lundström) cytospecies F larvae from Mobile County, Alabama (Nelder, unpublished data).

During experiments in which rotifers

Table 1. Mean relative abundance of *Smittium megazygosporum*, *S.* near *typhellum*, and *S. morbosum* in the hindgut of larval *Simulium vittatum* cytospecies IS-7.

Trichomycete Species	Mean Relative Abundance % (n larvae examined)[a]	
	Rotifers Absent in Container	Rotifers Present in Container
S. megazygosporum (experiment 1)[b]	19.2 (47)	0.6 (33)*
S. n. *typhellum* (experiment 2)	14.7 (20)	1.2 (10)*
S. n. *typhellum* (experiment 3)	8.4 (27)	0.0 (19)
S. morbosum (experiment 3)	26.8 (20)	9.9 (10)

[a] Relative abundance = percentage of ocular grid squares containing hyphae. For each experiment data were analyzed using a *t*-test on arcsine transformed percents; however, raw data are presented for comparative purposes. For each species, an asterisk indicates a significant difference ($p < 0.05$) in hyphal abundance. No test could be performed on *S.* n. *typhellum* from the second experiment.

[b] Each experiment had a total of 12 replications.

were found, black flies had been dosed with trichospores of either *Smittium megazygosporum* Manier and Coste, *S.* near *typhellum* Manier and Coste, or *S. morbosum* Sweeney. In black fly larvae taken from rotifer-infected containers, the relative abundance of both *S. megazygosporum* and *S.* near *typhellum* in the hindgut was significantly lower ($p < 0.05$) than in larvae taken from rotifer-free containers (Table 1). Trichospores of these fungi are well within the size range of particles fed on by bdelloids (i.e., 4–17 μm; Gilbert 1985). Accordingly, bdelloids might have reduced the number of trichospores passing to the hindgut, which in turn reduced the number of attached thalli.

We suspect the nature of the relationship between bdelloid rotifers and black fly larvae is one of accidental commensalism. Under this scenario, larval black flies are not effected by the rotifers; however, the rotifers use trichospores as a food source. Clearly, the exact nature of the relationship between black flies and rotifers warrants further investigation.

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